

Excretion and Tissue Distribution of 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin in the Rat

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The compound, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), is highly toxic. The LD₅₀ for male and female rats given a single oral dose is 23 and 45 $\mu\text{g/kg}$, respectively (1). Adverse effects have been observed in a teratology study in which pregnant rats were given oral doses of 0.125–2.0 $\mu\text{g/kg-day}$ TCDD from day 6 through day 15 of gestation (2). The adverse effects were increased fetal mortality, early and late resorptions and intestinal hemorrhage in the fetuses. No adverse effects were noted at the 0.03 $\mu\text{g/kg-day}$ level.

In humans and rabbits, contamination of the skin with TCDD produces chloracne-like lesions (3, 4). This disease is characterized by the appearance of hyperkeratosis, papules, comedones and cysts.

There is no available information on the absorption, excretion or tissue distribution of TCDD in animals. Therefore, this study was done to determine the excretion and tissue distribution of radioactivity derived from TCDD-¹⁴C following a single oral dose of the labeled compound.

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Methods

Animals

Male Spartan strain Sprague-Dawley rats weighing 165–210 g were used. The rats were acclimated to the environment of the metabolism cages 5 days prior to dosage. Food and water were provided *ad libitum* throughout the experiment.

¹⁴C-Tetrachlorodibenzo-*p*-dioxin

Uniformly labeled TCDD-¹⁴C was synthesized at the Radiochemistry Research Laboratory of The Dow Chemical Company. The specific activity was 2.8 $\mu\text{Ci/mg}$. Mass spectrometric and gas-liquid chromatographic analyses of the TCDD-¹⁴C sample indicated a purity of 93.3 and 95.0%, respectively.

Dosage

TCDD was dissolved in acetone. Subsequently, one part of the acetone solution was added to and mixed with nine parts of USP corn oil. The acetone-corn oil solution of TCDD was given to rats in 5 ml/kg amounts by intubation. This volume of the solution provided a dose of 50 $\mu\text{g/kg}$ and 0.14 $\mu\text{Ci/kg}$ TCDD-¹⁴C.

Sample Collection

After administering the solution containing TCDD-¹⁴C, the rats were placed in all-

glass Roth metabolism chambers which were equipped for separate collection of urine, feces, and expired air. The CO_2 in the exiting air stream was trapped by bubbling it through a 3:7 ethanolamine-2-methoxyethanol mixture.

Radioactivity Analysis

Samples of urine, feces, and tissues were oxidized to $^{14}\text{CO}_2$ by combustion and analyzed for radioactivity (5). By using this method, the recovery of radioactivity from samples spiked with ^{14}C was $95 \pm 5\%$. To determine the radioactivity expired as CO_2 , 5-ml aliquots of the solution used to trap the CO_2 were added to 15 ml of a scintillation counting solution containing 4 g 2,5-diphenyl-oxazole (PPO) and 0.1 g 1,4-bis-2-(5-phenyl-oxazolyl) benzene (POPOP) per liter of 1:1 toluene-2-methoxyethanol. Samples were counted for radioactivity in a Nuclear Chicago Mark II liquid scintillation counter, and quench corrections were made by use of the internal standard technique.

Results

The percentage of the total dose of radioactivity excreted daily in the feces, urine, or expired air over a 21-day period following a single oral dose of TCDD- ^{14}C is shown in Figure 1. Approximately 30% of the ^{14}C activity was excreted in the feces during the first 48 hr. Most of this probably represents unabsorbed TCDD- ^{14}C . Over the remaining 19 days, 1–2% per day of the ^{14}C activity was excreted in the feces. A total of $53.2 \pm 3.8\%$ of the administered dose was excreted via the feces over the 21-day period. The total cumulative amount excreted in the urine and expired air was $13.2 \pm 1.3\%$ and $3.2 \pm 0.1\%$, respectively.

To determine the overall rate of clearance of ^{14}C administered as TCDD from the body, the total cumulative amount of ^{14}C excreted in feces, urine, and expired air at the end of each day was subtracted from the total dose administered to the animal. These values, representing the percentage of the total dose remaining in the animal at the end of each day, were then plotted

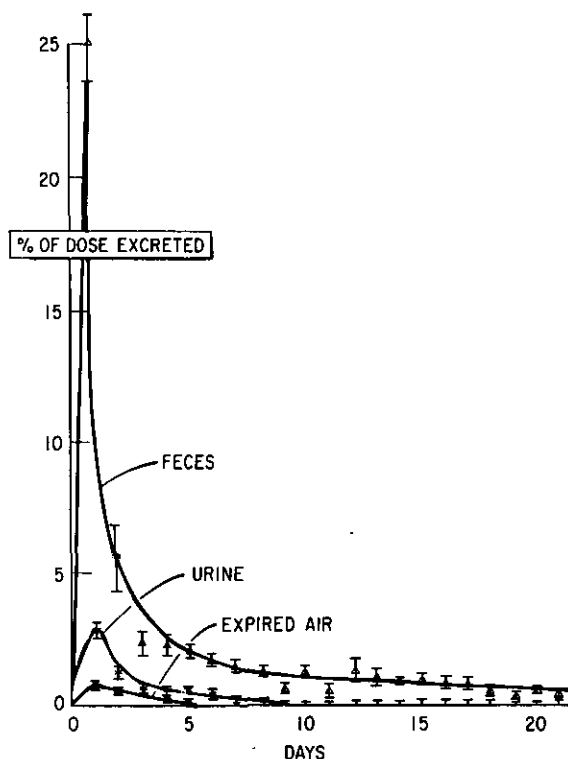


FIGURE 1. Excretion of ^{14}C activity by rats following a single oral dose of $50 \mu\text{g/kg}$ ($0.14 \mu\text{Ci/kg}$) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Each point represents the mean \pm SE for three rats.

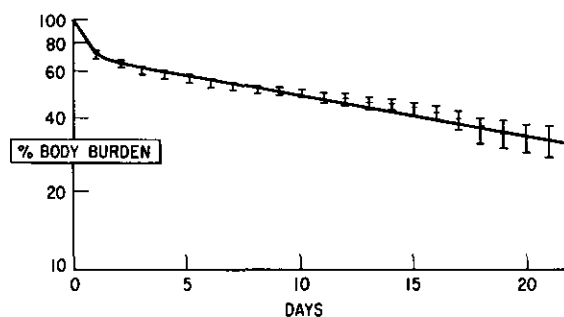


FIGURE 2. Clearance of ^{14}C activity from the body of rats given a single oral dose of $50 \mu\text{g/kg}$ ($0.14 \mu\text{Ci/kg}$) 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Each value represents the mean \pm SE for three rats.

semilogarithmically as a function of time (Fig. 2). Except for the first 2 days following administration, the clearance of ^{14}C activity from the body followed apparent first-order rate kinetics. The half-life for

clearance, $t_{1/2}$, was 17.4 ± 5.6 days. As previously indicated, it was assumed that the relatively large amount excreted during the first 2 days had not been absorbed. Therefore, these values were not used in calculating the clearance rate.

Analyses of tissues indicated that the ^{14}C activity derived from TCDD and/or its breakdown products was located chiefly in the liver and fat (Table 1). The percentage of the dose per gram of liver 3, 7, and 21 days following administration was 3.18, 4.49 and 1.33%/g, respectively. Comparable values for fat were 2.60, 3.22, and 0.43%/g.

Smaller concentrations of ^{14}C activity were found in other tissues: muscle, testes, lungs, heart, skin, spleen, stomach, pancreas, brain, bone, kidneys, and adrenals (Table 2). Standard errors as large as the mean suggest that some of the values presented in Table 2 may be a result of experimental error: adrenals, 3 days; bone, 7 days; spleen, 21 days; pancreas, 21 days. Radioactivity exceeding background in these tissues at the indicated time was detected in only one of three rats.

The value given in Table 2 for adrenals 21 days following administration suggests that this tissue may concentrate TCDD- ^{14}C and/or a metabolite. This observation is very likely due to experimental error. The disintegrations per minute (dpm) above background for this tissue were only 17, 29, and 90. Since the total amount of tissue available for analysis was less than 20 mg, the multiplication factor may have magnified the error manyfold.

Total recovery of the administered ^{14}C activity was determined for those rats used in the 21-day experiment. The ^{14}C activity remaining in the unused carcass was determined by analyzing an aliquot of a homogenate of the remaining carcass. The recovery was $96.8 \pm 3.0\%$.

Discussion

In the study reported herein, the tissue distribution and excretion of ^{14}C activity have been evaluated in rats following a single oral dose of TCDD- ^{14}C . Almost 30% of the

Table 1. ^{14}C activity expressed as percentage of dose per gram in the liver and fat of rats 3, 7, and 21 days following a single oral dose of TCDD- ^{14}C .^a

Tissue	Activity, %/g ^b		
	3 days	7 days	21 days
Liver	3.18 ± 0.21 (47%) ^c	4.49 ± 0.62 (45%) ^c	1.33 ± 0.70 (11%) ^c
Fat	2.60 ± 0.48	3.22 ± 0.63	0.43^d

^a Dose: 50 $\mu\text{g/kg}$ (0.14 $\mu\text{Ci/kg}$); 3 rats/observation.

^b Mean \pm standard error.

^c Percentage of the total dose found in the entire liver.

^d Mean for 2 rats.

Table 2. ^{14}C activity expressed as percentage of dose per gram in various tissues of rats 3, 7, and 21 days following a single oral dose of TCDD- ^{14}C .^a

Tissue	Activity, %/g ^b		
	3 days	7 days	21 days
Muscle	0.38 ± 0.01	0.21 ± 0.05	0.20 ± 0.12
Testes	0.38 ± 0.03	0.36 ± 0.10	0.11 ± 0.09
Lungs	0.27 ± 0.02	0.39 ± 0.14	0.06 ± 0.05
Heart	0.20 ± 0.03	0.40 ± 0.16	0.09 ± 0.05
Skin	0.19 ± 0.10	0.19 ± 0.10	0.09 ± 0.04
Spleen	0.15 ± 0.02	0.95 ± 0.53	0.22 ± 0.22
Stomach	0.16 ± 0.05	0.10 ± 0.00	0.02 ± 0.02
Pancreas	0.11 ± 0.06	0.16^c	0.16 ± 0.16
Brain	0.06 ± 0.00	0.13 ± 0.09	0.01 ± 0.01
Bone	0.09 ± 0.03	0.42 ± 0.42	0.08 ± 0.08
Kidneys	0.00^d	0.34 ± 0.17	0.00^d
Adrenals	0.79 ± 0.79	0.02 ± 0.02	3.69 ± 1.77^e

^a Dose: 50 $\mu\text{g/kg}$ (0.14 $\mu\text{Ci/kg}$); 3 rats/observation.

^b Mean \pm standard error.

^c Mean of 2 rats.

^d No activity above background in all 3 rats.

^e This large value may be an experimental error. The activity above background for the adrenals of the three rats was 17, 29, and 90 dpm. Since the total amount of tissue was less than 20 mg, the multiplication factor may have magnified the error manyfold.

dose administered was eliminated via the feces during the first 48 hr following treatment. The excretion of ^{14}C activity via the feces after the first 48 hr ranged from 1 to 2%/day. It appears that TCDD is incompletely absorbed from the gastrointestinal tract. The ^{14}C activity derived from the ab-

sorbed TCDD-¹⁴C also is excreted mainly via the feces.

Once absorbed in the body, most of the ¹⁴C activity derived from TCDD-¹⁴C is localized in the liver and fat. The data suggest that the level in these tissues is approximately 10-fold that in other tissues. The ¹⁴C level in liver and fat seemed to increase between 3 and 7 days following administration; however, the ¹⁴C activity in liver and fat decreased more between 7 and 21 days than would have been predicted on assuming that the rate of clearance from these tissues would be equal to the rate of clearance from the body. Between days 7 and 21, the ¹⁴C level in muscle remained essentially unchanged. Therefore, redistribution of TCDD or metabolites of TCDD may have been occurring. The apparently high level in the adrenals 21 days after administration results probably from experimental error.

The dose of TCDD given to the male rats used in this study, 50 µg/kg, was approximately twice the LD₅₀ (23 µg/kg). This large dose was necessitated because of the specific activity of the TCDD-¹⁴C used. Rats lost weight, and their physical condition was poor; this typifies the insidious response to TCDD (1). Survival of the rats for 21 days was not totally unexpected, because in previous studies on the lethality of TCDD deaths frequently occurred 20 days or more following a single oral dose of similar magnitude

(1). With doses that do not induce untoward effects, the compound may be excreted at a different rate.

The results do not differentiate between ¹⁴C activity derived from TCDD and that of possible metabolites. However, small amounts of ¹⁴C activity were detected in the expired air and urine within the first 10 days following administration. This is evidence that some metabolic alteration or breakdown of TCDD occurs.

Acknowledgement

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REFERENCES

1. Schwetz, B. A., et al. Toxicology of chlorinated dibenzo-*p*-dioxins. In: *Advances in Chemistry Series 120*, American Chemical Society, Washington, D.C., 1973, p. 55.
2. Sparschu, G. L., Dunn, F. L., and Rowe, V. K. Study of the teratogenicity of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *Food Cosmet. Toxicol.* 9: 405 (1971).
3. Kimmig, J., and Schulz, K. H. Occupational chloracne caused by aromatic cyclic ethers. *Dermatologia* 115: 540 (1957).
4. Jones, E. L., and Krizek, H. A technic for testing acnegenic potency in rabbits, applied to the potent acnegen, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *J. Invest. Dermatol.* 39: 511 (1962).
5. Smith, G. N., et al. Simple apparatus for combustion of samples containing C¹⁴-labeled pesticides for residue analyses. *Agr. Food Chem.* 12: 172 (1964).